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Changes in Morphology of a Rat Adenocarcinoma Model Induced by Intravenous Seeding into the Lung

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SUMMARY

Cell cultures from R3230AC rat mammary tumor, when injected i. v. into syngeneic F344 rats, regularly produce multiple lung tumour foci within 10–14 days. When explants from these lung nodules were cultured *in vitro* and re-injected as cell suspensions five times, their morphology changed completely from the original pure adenocarcinoma to squamous cell carcinoma. This change appears to be stable for over 12 subsequent subcutaneous passages.

Our results suggest that the inherent genetic instability of the tumor together with a possible selection process of cells with metastatic potential explain the change in tumor phenotype observed.

Key words: Rat mammary adenocarcinoma, Lung metastases, Change of histological type

INTRODUCTION

Neoplasms include subpopulations of cells with diverse phenotype, that co-exist and interact. Characteristics of tumor cells which may change over time, both *in vitro* and *in vivo,* include morphological features, response to hormones and therapeutic modalities and a variety of biochemical parameters. The cause of this heterogeneity is unknown and such explanations as inherent genetic instability or selective advantage are speculative (1). The possibility also exists of true conversions from one to another, developmentally unrelated, cell type (3, 10). The acquisition of increased metastatic potential appears to be related to variability in expression of phenotypes (5).

In this study, we subcultured a well-established adenocarcinoma in the rat, which has been considered a very stable model. We then repeatedly transferred

metastatic foci into cell cultures and back to the animal host, which led to the establishment of a metastatic cell line with an apparently stable new morphology.

MATERIALS AND METHODS

Tumor Model

The R3230AC rat adenocarcinoma (obtained courtesy of Dr. A. Bogden, EG and G Mason Research Institute, Worcester, Mass.) was maintained in female F344 rats weighing 130–150 g, by subcutaneous implants of Stadie slices. All animals used in the following experiments were of the same batch, maintained under identified feeding and housing conditions.

Cell Cultures

Explants obtained from a subcutaneously grown tumor tissue sample were initially cultured in 25 m*l* Falcon flasks. Monolayer cultures derived from these explants were maintained in 75 m*l* Falcon flasks in RPMI-1640 medium with antibiotics and supplemented with 10% fetal bovine serum. The cultures were incubated in 5% CO_2 and 95% air at 37 ℃. After 72–96 hours, the monolayer achieved confluency. Single cell suspensions were prepared by trypsinization (with 0.05% Trypsin and 0.01% EDTA). Cells were counted in a haemocytometer and viability checked by the trypan blue exclusion method.

Pulmonary Seeding and Harvesting

A saline suspension of 1×10^6 viable cells from the cell culture was injected into the tail veins of five female F344 rats. Fourteen days later, the animals were sacrificed by cervical dislocation. A random chosen pulmonary neoplastic nodule was harvested at autopsy, teased, and grown in culture as described for the original tumor. This cycle of cell culture, seeding and harvesting was repeated five times using groups of five rats for each passage.

Subcutaneous Implants

For subcutaneous growth, $1\text{–}2\times10^7$ cultured cells of both populations, that is from the original subcutaneous solid tumor and the line derived from lung tumor nodules were injected s. c. in the back of the rats.

Morphological Examination

Samples of subcutaneous nodules and whole lung slices obtained after the first and fifth seedings with tumor cells were fixed in 4% buffered formaldehyde, embedded in paraffin and 4 μ sections were stained with haemoatoxylin-eosin. For electron microscopy 1 mm cubes from subcutaneous and lung tumor nodules (5th

generation) were fixed in 4% glutaraldehyde, embedded and cut in Epon, and thin sections were stained with uranyl acetate and lead citrate for electron microscopic examination.

RESULTS

The original tumor in subcutaneous explants (and first generation pulmonary seedings) is a poorly differentiated adenocarcinoma, growing in large solid nests with occasional small glandular lumina (Fig. 1). Ultrastructurally, the tumor cells are joined by junctional complexes with infrequent desmosomes to form microlumina lined with microvilli (Fig. 3). No bundles of intermediate filaments are seen. In our hands, this has been confirmed for 30 subcutaneous passages.

In the fifth generation of pulmonary nodules, the original glandular pattern had changed completely to that of a keratinizing squamous cell carcinoma. The tumor cells were arranged in concentric layers to envelop small keratin pearls had contained keratohyaline granules (Fig. 2). Ultrastructurally, the tumor cells had no microvilli, interdigitated with neighbouring cells by broad cytoplasmic projections and were connected by frequent desmosomes (Fig. 4). Aggregates of intermediate filaments were found in the cytoplasm (Fig. 5). No cells with glandular features were found in any of these fifth generation nodules, either by light or electron microscopy.

The squamous neoplastic characteristics persisted for at least 12 passages of subcutaneous inoculations of subcultures from this pulmonary cell nodule line in all portions of all tumors as well as in the lung foci after i. v. seeding.

DISCUSSION

The R3230AC rat mammary tumor model has been used for over 20 years with little change in its original characteristics (morphology, hormone responsiveness, steroid receptors, doubling time, etc.). It can be cultured *in vitro* and intravenous injections of 10^6 viable cells, invariably produce multiple neoplastic lung foci within 10–14 days (6). In our study, the light microscopic characteristics of a pure adenocarcinoma were found both in s. c. propagated tumor and lst generation lung foci, and confirmed by electron microscopy for both types of sample. Only typical glandular cells with microlumina, microvilli and infrequent desmosomes were found. The five alternating *in vivo–in vitro* cycles, however, completely transformed the tumor. After the fifth passage the pulmonary foci showed obvious features of keratinizing squamous cell carcinoma, ultrastructurally characterized by numerous desmosomes and intermediate filament bundles in aggregates. A pure squamous cell carcinoma morphology has since persisted in the offspring of these pulmonary foci after 12 passages in subcutaneous implants.

The long stability of our tumor model appears to confirm Willis' statement that " Each tumor has its own peculiarities of structure and behaviour, which it maintains largely unchanged even through a long and complicated metastatic career " (9). This epitomizes a century long study of tumors and has proved its worth in the morphological diagnosis and clinical management of neoplasms. The concept of tumor heterogeneity that has emerged during the last two decades, and the results of the present study, seem opposed to Willis' statement. Genetic instability, postulated to be an inherent feature of all malignant tumors, maintains a heterogeneous tumor population by the constant emergence of new variants. What factors keep these two paradoxical aspects of tumor nature in balance is as yet poorly understood, although tumor characteristics including morphology have been altered experimentally. Thus, Dunning used testosterone to induce the transformation of RC3230AC adenocarcinoma into a keratinizing squamous cell carcinoma (4), and Ramaekers *et al.* reported a newly developed vimentin production in carcinomatous cells dispersed in ascitic or pleural fluid (7). In contrast to the permanent phenotypic change achieved in our experiment, the ability to produce vimentin was transient and entirely dependent on the continuing presence of the initiating stimulus (ascitic or pleural fluid) (8). A change in degree of differentiation (grade) has been reported to occur following passaging of the mammary adenocarcinomas (including the RC3230AC line) in nude athymic mice (2).

Two possible mechanisms for the change observed in our study might be considered: 1. Squamous cells in the original tumor were so infrequent as to be missed by light and electron microscopy, but had a higher capability to grow in lung tissue and were selected by repeated passage; 2. The lung environment stimulated an inherent potential of the tumor cells for squamous differentiation, with a selective process favouring the growth of cells with the best-developed squamous potential. The absence of demonstrable squamous cells both in the original tumor and in the first generation seedings seems to favour the second explanation.

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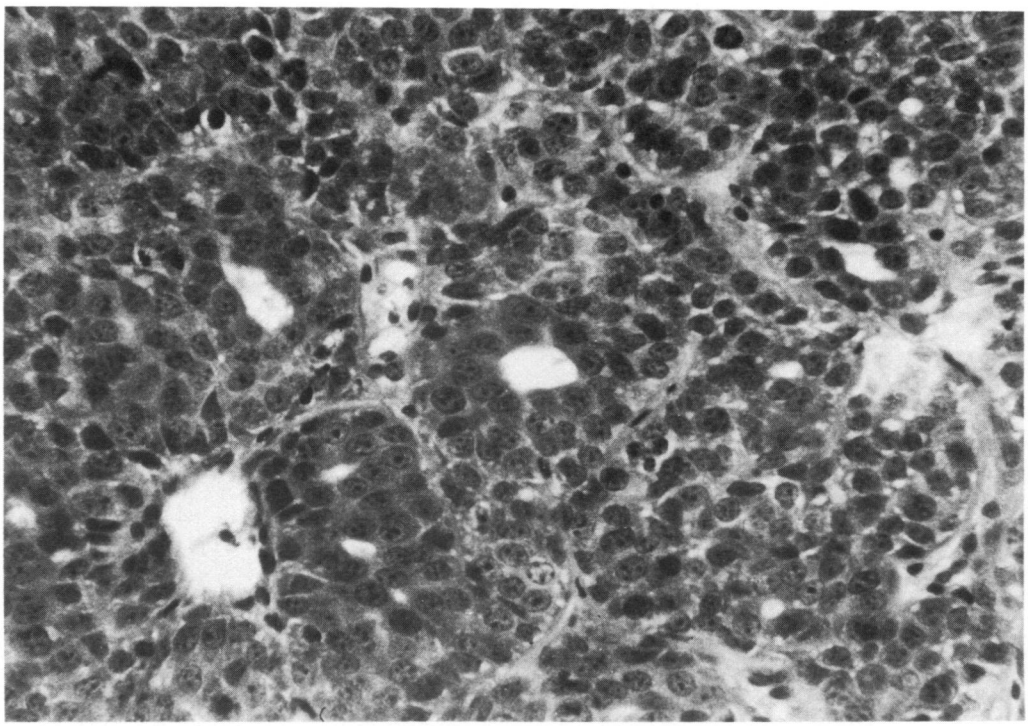

Fig. 1 Original adenocarcinoma (RC3230AC) from subcutaneous implant. HE, 400×.

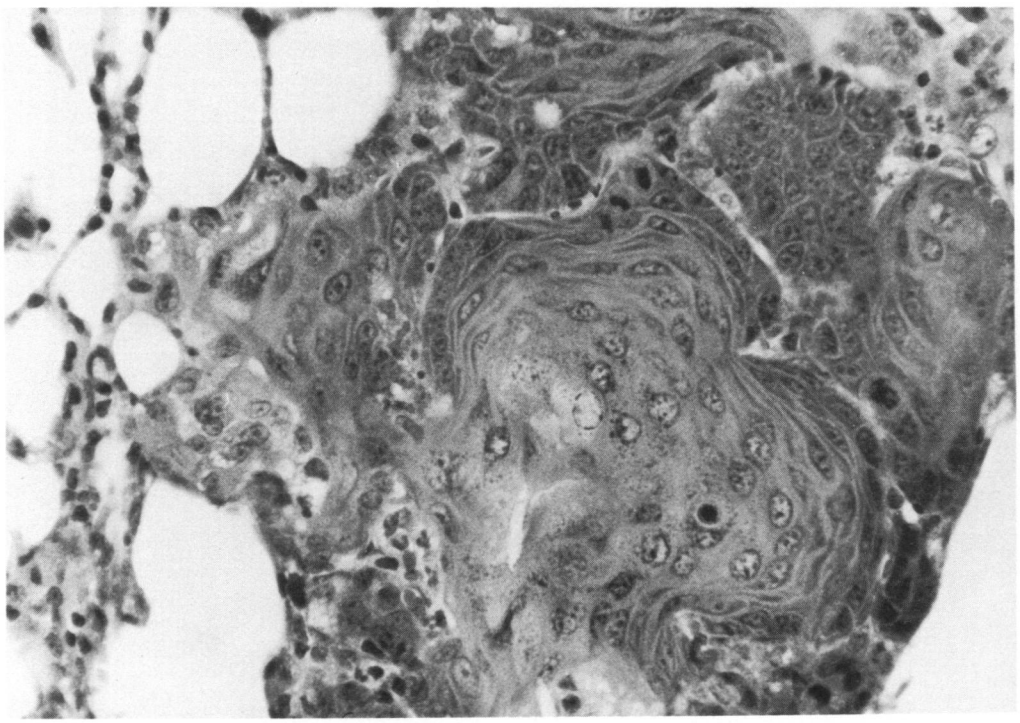

Fig. 2 Pulmonary neoplastic nodule (5th passage) showing morphology of squamous cell carcinoma. HE, 400×.

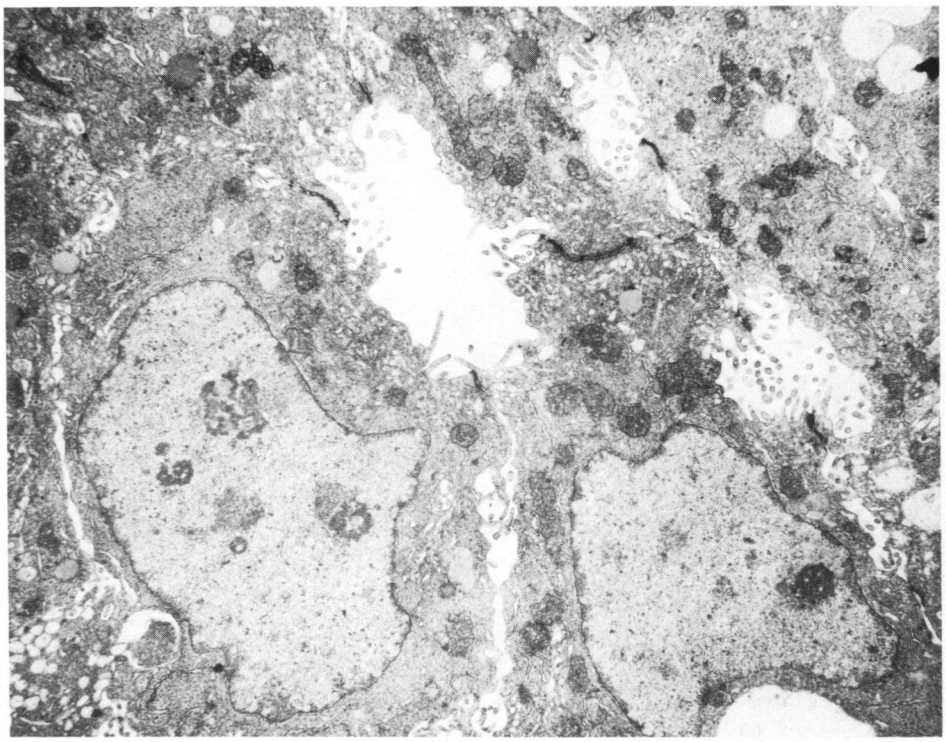

Fig. 3 Ultrastructure of original adenocarcinoma (RC3230AC) from subcutaneous implant. 15000×.

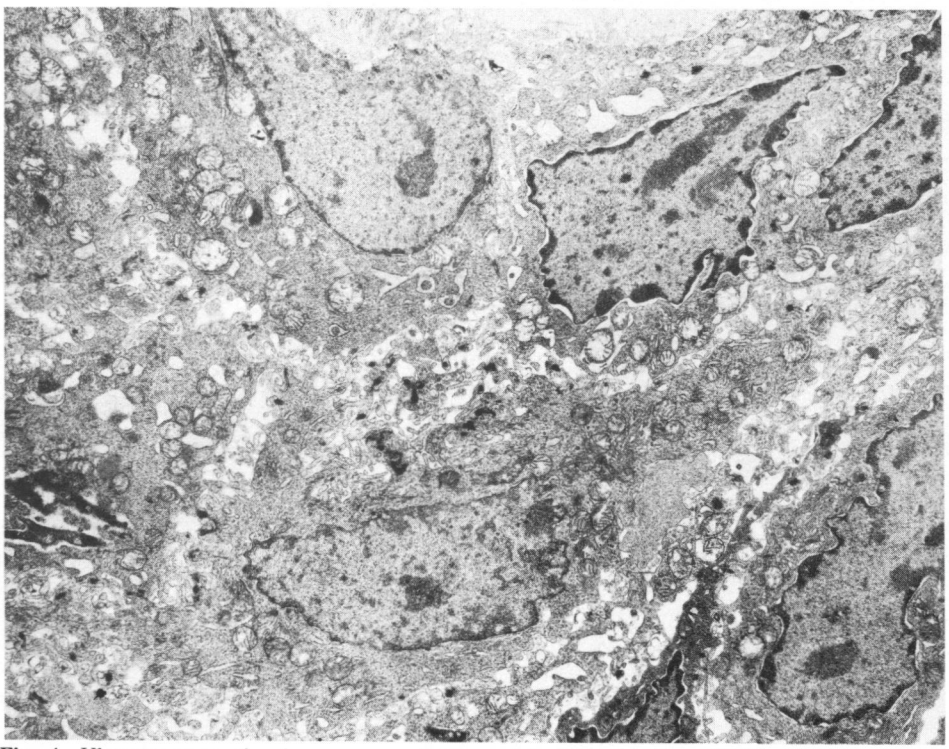

Fig. 4 Ultrastructure of pulmonary neoplastic nodule (5th passage). 11000×.

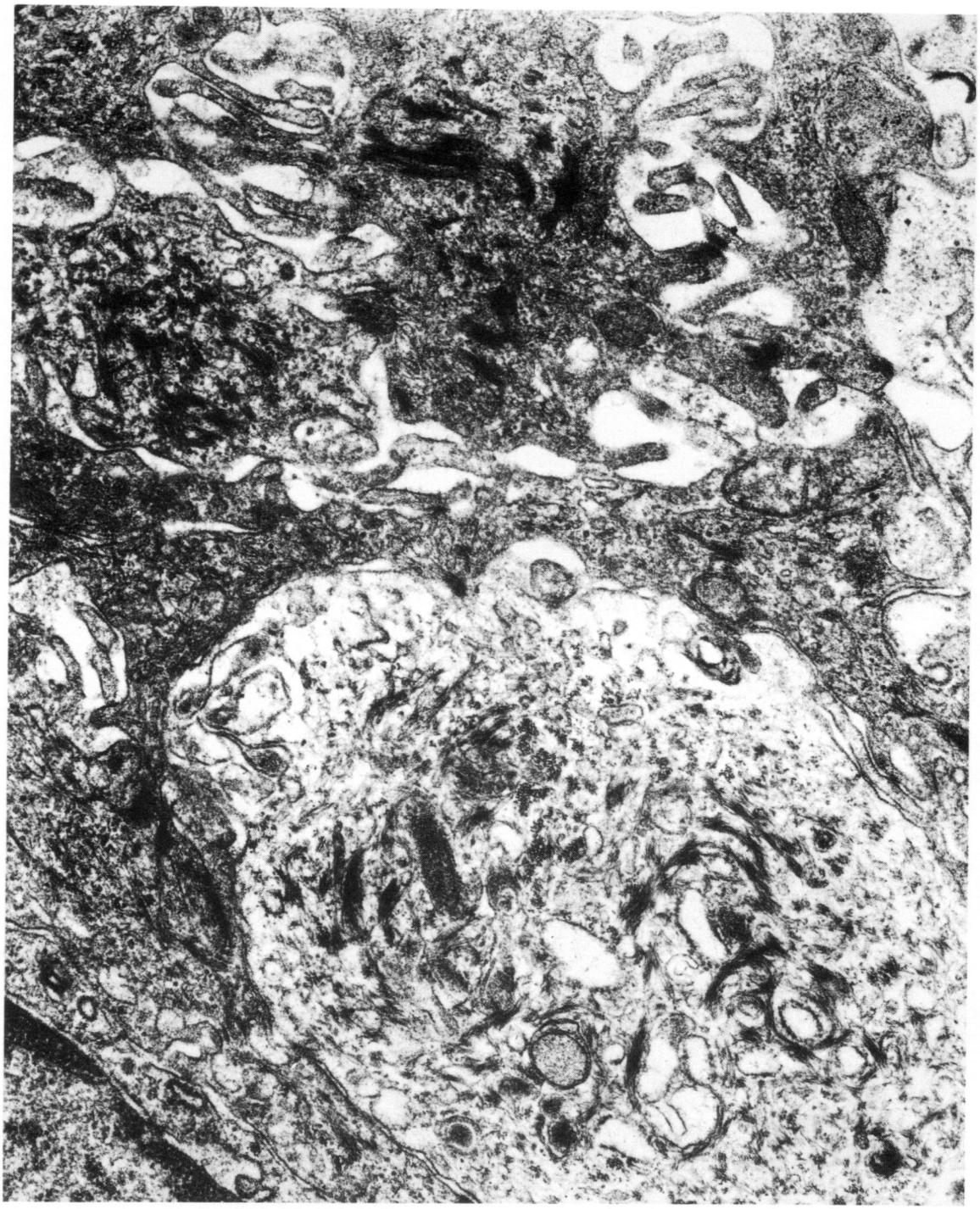

Fig. 5 Ultrastructural detail from pulmonary neoplastic nodule (5th passage) showing aggregated bundles of intermediate filaments. 25000×.